INTRODUCTION

Plants have played a vital role in medicine since ancient times and continue to be important in the modern healthcare system. In developing countries, almost 80% of the population relies on traditional medicines derived from plants for their healthcare needs (Salmeron *et al.,* 2020). Around 10% of vascular plants are used for medicinal purposes, with over 1,340 species exhibiting therapeutic properties. Significantly, 74% of bioactive compounds derived from plants have been discovered through traditional ethnomedicinal knowledge, highlighting the value of indigenous practices in guiding modern pharmaceutical research and development (Vaou *et al.,* 2021).

Medicinal plants are rich sources of secondary metabolites such as terpenoids, phenols, flavonoids, alkaloids, tannins and aromatic compounds. These bioactive compounds are not only essential for plant growth, but are also crucial for defense and adaptation. These key compounds are widely used in pharmaceuticals and other industries (Pant *et al.,* 2021). Traditional drug discovery, which primarily focuses only on single targets, is now evolving into multi-target approaches (network pharmacology), especially for diseases like cancer and diabetes that involve complex interactions (Noor *et al.,* 2022).

Rhododendron arboreum Sm. Overview

Rhododendron arboreum Sm. a species from the Ericaceae family, is well-known for its medicinal properties. It is found across many regions in India, including Arunachal Pradesh, Jammu and Kashmir, Manipur, Meghalaya, Sikkim, Uttarakhand and Himachal Pradesh (Mehta *et al.,* 2023).

Plant Description

The name *Rhododendron* is derived from Greek, where 'Rhodon' means "rose" and 'dendron' means "tree" (Kumar *et al.,* 2020). This is an evergreen tree, can grow up to 15-20 meters tall and has leathery, lance-shaped leaves with a dark green upper surface and rusty-brown underside with bell-shaped flowers, which bloom in clusters of red, pink or white.

R. arboreum is traditionally used to cure stress, headaches, diarrhea and altitude sickness. It is also culturally significant, in making pickles, ropes and even fuelwood (Sharma *et al.,* 2020).

Gastric cancer is a highly lethal disease with poor global survival rates. Annually, most new cases are reported in Asian and South American nations, with around 27,000 new diagnoses in the USA in 2020 (Sexton *et al.,* 2020). In 2018, it was the fifth

most diagnosed cancer, responsible for 8.2% of all cancer-related deaths worldwide, making it the third leading cause of cancer death, following lung (18.4%) and colorectal cancers (9.2%). The global incidence and mortality rates in 2018 were 11.1 and 8.2 per 100,000 people respectively (Thrift and El, 2020).

While gastric cancer incidence is higher in countries with a high Human Development Index (HDI), mortality rates are lower in these countries compared to medium or low HDI nations. This disparity is attributed to better healthcare access, early detection and treatment options in higher HDI regions. In 2020, Eastern and Southeastern Asia, known for prevalent virulent *Helicobacter pylori* strains, accounted for nearly two-thirds of the world's gastric cancer cases, with Eastern Asia having the highest incidence rate of 22.4 per 100,000 people (Thrift *et al.*, 2023).

Plants produce antioxidants like flavonoids, phenolic acids and alkaloids that scavenge free radicals, protecting cells from oxidative stress. The role of antioxidants in preventing oxidative damage is crucial, as they neutralize excess reactive oxygen species (ROS), which can cause oxidative stress and damage to biomolecules such as lipids, proteins and nucleic acids. ROS are generated naturally in metabolic processes and can be exacerbated by external factors like pollution, smoking or radiation. Antioxidants, both enzymatic (like glutathione) and non-enzymatic (e.g., vitamins, natural compounds) mitigates these effects by stabilizing ROS, preventing cellular damage and reducing disease risks (Rammohan *et al.*, 2020; Flieger *et al.*, 2021).

In cancer therapeutics, animal models like C57BL/6 mice are essential for studying tumor biology and testing potential treatments, as they offer genetic uniformity, immune system relevance and well-established gastric cancer models. These models enable researchers to explore therapeutic strategies, including cancer vaccines, immune responses and gene-targeting treatments (Mak *et al.*, 2014; Benavides *et al.*, 2020). The reliability of C57BL/6 mice in mimicking human gastric cancer and their extensive historical use make them a valuable tool in preclinical cancer research (Chen *et al.*, 2022).

Network pharmacology has emerged as a multidisciplinary approach, combining systems biology, bioinformatics and pharmacology to explore the complexity of drug-target interactions. Unlike traditional pharmacology, which focuses on individual drug-target relationships, network pharmacology emphasizes the modulation of multiple targets within biological systems, offering a holistic view of drug actions, disease mechanisms and therapeutic strategies, especially for complex diseases like cancer and cardiovascular disorders.

A key tool in network pharmacology is the STRING database, which predicts and visualizes protein-protein interaction (PPI) networks, helping to understand protein collaboration and identify potential therapeutic targets. Cytoscape is another vital database which helps in analyzing the molecular networks visually. It aids in exploring network topology and identifying druggable nodes that could impact entire molecular networks.

Functional enrichment analyses, including Gene Ontology (GO) and KEGG pathway enrichment, further enhance the interpretation of molecular networks by identifying over-represented biological processes and pathways related to diseases or drug responses. GO enrichment provides insights into relevant biological processes, while KEGG helps to map the known biological pathways onto PPI networks, identifying potential targets for drug discovery.

The integration of tools like STRING, Cytoscape, GO and KEGG in network pharmacology shifts the focus from single molecular targets to broader networks, providing a strong framework for multi-target drug discovery, personalized medicine and drug repurposing. This approach is particularly valuable in addressing complex diseases and enhancing therapeutic efficacy by targeting multiple proteins or pathways simultaneously.

In gastric cancer, the Epidermal Growth Factor Receptor (EGFR) tyrosine kinase pathway plays a critical role in tumorigenesis and disease progression. When activated by ligands like Epidermal Growth Factor (EGF), EGFR triggers downstream signaling cascades, including the Ras/MAPK and PI3K/Akt pathways, which regulate cell proliferation, survival and migration (Uribe *et al.,* 2021; Cuesta *et al.,* 2021). Dysregulation of this pathway, such as EGFR overexpression or mutations, leads to uncontrolled cell growth, metastasis and resistance to apoptosis, worsening the prognosis in gastric cancer (Lei *et al.,* 2022).

Targeted therapies, like cetuximab and erlotinib, have been developed to inhibit EGFR activity, but resistance often emerges due to mutations or activation of alternative pathways (Romaniello *et al.,* 2020). Hence, understanding EGFR signaling is crucial for optimizing gastric cancer treatments.

In silico methods, particularly molecular docking and molecular dynamics (MD) simulations, are invaluable for identifying potential therapeutic compounds against gastric cancer (Kapetanovic, 2008). These approaches help visualize protein-ligand interactions and predict ADME properties, improving the drug design process (Rao and Srinivas, 2011).

Objectives of the Study

Rhododendron arboreum has emerged as a subject of interest due to its traditional medicinal applications. This plant, known for its therapeutic properties in traditional medicine, is being studied for its potential role in cancer treatment. Exploring its bioactive compounds, which demonstrate promising anticancer effects. These compounds are believed to interfere with cancer cell processes, potentially inhibiting tumor growth and metastasis. The proposed research work focuses on the anticancer potential of *R. arboreum* extracts through *in vitro* studies and *in vivo* models; which aims to harness the findings for future therapeutic applications against various types of cancer. The proposed research work comprises four distinct phases, each with specific objectives outlined below.

- ✓ To study the phytochemical composition of *R. arboreum* Sm. leaf and flower extracts
- ✓ To determine the antioxidant and antiproliferative effects of *R. arboreum* Sm. extracts (leaves and flowers) on gastric cancer cells
- ✓ To decipher the prominent targets associated with gastric cancer through network pharmacology and elucidate the molecular interaction of bioactive compounds with the identified targets
- ✓ To validate the efficacy of *R. arboreum* Sm. leaves and flowers extract using gastric cancer induced mice model (C57BL/6)

Research Gap

Although there is a considerable amount of literature dealing with the phytochemical composition, antioxidant and anti-inflammatory properties of *Rhododendron arboreum*, there is still a significant research gap regarding its application in living biological systems. So far, no *in vivo* studies have been conducted with this plant, particularly in relation to gastric cancer. This gap emphasizes the need for further research to explore the potential therapeutic effects of *R. arboreum* in the treatment of gastric cancer, as studies to date have mainly focused on *in vitro* evaluations and general biological activities.

MATERIALS AND METHODS

Medicinal plants have always been an essential part of human health and offer natural remedies for a variety of ailments. They contain bioactive compounds such as alkaloids and flavonoids that have therapeutic properties. In all cultures, these plants are valued for their traditional healing practices and offer treatments for ailments ranging from common cold to chronic diseases. The present research aims to discover new applications and optimize their potential benefits, highlighting their continued importance to medicine and wellbeing.

The leaves and flowers of *Rhododendron arboreum* were collected from Pedung village in West Kameng district of Arunachal Pradesh, India and authenticated at BSI (Botanical Survey of India) Coimbatore. Tamil Nadu. (No.: BSI/SRC/5/23/2021/Tech on 25/02/2021)

The collected leaves and flowers were completely washed and dried in the shade for a fortnight. The dried samples were powdered separately and stored in an airtight container for further analysis. 20 g of the powdered leaves and flowers were soaked in 200 ml of aqueous, methanol, ethanol, acetone, chloroform and petroleum ether in a separate conical flask and incubated for 72 hours at room temperature and then in incubator shaker at 150 rpm, then filtered and further concentrated by rotary vacuum evaporator at 125 rpm in water bath (~48^{o}C).

PHASE I

1. Leaf and Flower extracts were tested for the presence of bioactive compounds using standard methods (Pandey and Tripathi, 2014; Dhawan and Gupta, 2017).
2. Quantitative analysis is done by the following standard methods
    - Estimation of flavonoids (Eom *et al*., 2016) using 10% aluminium chloride solution and potassium acetate solution.
    - Estimation of total carbohydrates (Hedge and Hofreiter 1962) using the anthrone reagent.
    - Determination of proteins: Lowry method (Waterborg, 2009) using alkaline copper solution and Folin-Ciocalteu reagent.
    - Determination of total tannins: Folin-Ciocalteu method (Folin and Ciocalteu, 1927) using sodium carbonate solution.

- Determination of total alkaloids: Bromocresol green method (Shamsa *et al.,* 2007) involving the reaction with bromocresol green reagent.
- Determination of total phenolics (Chedea and Pop, 2019), using the Folin-Ciocalteu reagent and sodium carbonate.

3. FTIR- Fourier Transform Infrared Spectroscopy (Lorenz, 2020) employing 100 mg KBr pellets as encapsulation in sample discs, which scanned the spectral range from 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}.
4. NMR- Nuclear Magnetic Resonance (Huang *et al.,* 2014) using Bruker AVANCE III instrument operating at 600.13 MHz and 25°C, ^{1}H NMR spectra were recorded with 16/64 scans, an acquisition time of 6.50 min per spectrum point, and a 5 s relaxation delay.
5. GC-MS-Gas Chromatography-Mass Spectrometry (Tayade *et al.,* 2022). Agilent GC-MS (CH-GCMSMS02) used with 8890 GC and 7000 GC/TQ. For interpretation of Gas Chromatography-Mass Spectrometry (GC-MS) peaks were matched with the National Institute of Standards and Technology (NIST) database.

PHASE II

1. Radical scavenging activity of *R. arboreum* Sm. methanolic extracts of leaf and flower were evaluated for the following assays.
   - DPPH Radical Scavenging assay (Blois, 1958).
   - ABTS radical scavenging assay (Re *et al.,* 1999).
   - H_2O_2- Hydrogen Peroxide Assay (Halliwell and Gutteridge 1985).
   - Lipid Peroxidation Assay (Repetto *et al.,* 2012).
   - FRAP Assay- Ferric reducing Antioxidant Power Assay (Benzie and Strain, 1996).
2. Cytotoxicity study using AGS cell lines- The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay by Cardile *et al.,* 2005 method.
3. For detecting cell death by flow cytometry, cells were stained with FITC Annexin V to identify early apoptosis and co-stained with propidium iodide (PI) to differentiate late apoptosis and necrosis.
4. Toxicity study- Brine Shrimp Lethality Assay (Hamidi *et al.,* 2014) was conducted to screen the toxicity of plant extracts by exposing *Artemia salina* to different concentrations for 24 hours and calculating the LC_{50} based on mortality rates.

5. Statistical analyses- The analyses were performed in triplicate to ensure accuracy, and the results were presented as mean values accompanied by their standard deviations. Subsequently, IC_{50} values for the antioxidant assays were calculated using the linear regression method. The experimental data underwent analysis using one-way factorial ANOVA, followed by the Tukey multiple range test at $\alpha = 0.05$.

PHASE III

1. *In vivo* studies on Docetaxel-Induced experimental mice model

Using the C57BL/6 model for *in vivo* cancer studies allows to observe treatment effects in living organisms, offering insights into tumor growth and therapy efficacy that cannot be gained from cell cultures alone. Therefore, the present study was conducted to evaluate the following parameters.

- Enumeration of Red Blood Cells (Raabe *et al.*, 2011).
- Differential Leukocyte Count (Nivedhita *et al.*, 2020).
- Estimation of Hemoglobin: Sahli's acid Haematin Method (Thakkar *et al.*, 2021).
- Estimation of Total Cholesterol (Jain *et al.*, 2020).
- Estimation of Triglycerides (Bertolo and Bogusz, 2022).
- High-Density Lipoprotein Cholesterol (Cole, 1997).
- Determination of Total Proteins (Waterborg, 2009).
- Determination of superoxide dismutase (SOD) (Kakkar *et al.*, 1984).
- Determination of Catalase (CAT) (Sinha *et al.*, 2022).
- Determination of Glutathione Peroxidase (GPx) (Rotruck *et al.*, 1973).
- Determination of reduced glutathione (GSH) (Ellman,1959).
- Determination of lipid peroxidation (LPO) (Ohkawa *et al.*, 1979).
- Myeloperoxidase Activity (MPO) (Wei and Frenkel, 1991).
- Histological Examinations (Ahmed *et al.*, 2015).

2. Statistical analysis- Values are expressed as mean ± SEM. Statistical significance (p) was calculated by one-way ANOVA followed by Dunnett's test (n=6); ns - non-significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, calculated by comparing treated groups with the control group.

PHASE IV

1. *In-silico* Studies- SWISS ADME to predict the pharmacokinetics, drug-likeness and potential ADME (absorption, distribution, metabolism and excretion) properties of small molecules in drug discovery.
2. Network Pharmacology to understand the interactions between selected drugs, targets and biological networks, helping to reveal multi-target mechanisms and predict therapeutic effects and potential side effects.
3. Molecular Docking to predict the interaction between a small molecule (ligand) and a target protein, to evaluate the binding affinity and stability of potential drug candidates.
4. Molecular Dynamic Simulation (MDS) to model the physical movements of atoms and molecules over time, allowing the study of molecular interactions, stability and conformational changes in biological systems.

RESULTS

PHASE I

Preliminary qualitative analysis revealed the presence of both primary and secondary metabolites in the aqueous, methanol, ethanol, acetone, chloroform and petroleum ether extracts of *R. arboreum* leaves and flowers.

In the quantitative analysis of *R. arboreum* leaves and flowers using methanol, ethanol and acetone as extraction solvents where leaf extracts showed flavonoid content of 117.1 mg/g, followed by alkaloids (75.95 mg/g), tannins (47.84 mg/g) and phenols (40.62 mg/g), whereas, flower extracts of flavonoids reaching the highest level (139.6 mg/g), followed by tannins (80.01 mg/g), phenols (47.16 mg/g) and alkaloids (32.31 mg/g). The results showed that methanol extracts had the highest concentrations of phytochemicals, confirming its effectiveness in extracting a wide range of polar compounds and facilitating additional investigations into the plants bioactive compounds for cancer prevention and treatment.

FTIR spectroscopic analysis of *R. arboreum* leaves and flowers extract revealed a diverse array of functional groups, indicating the presence of various bioactive compounds. The analysis highlighted significant functional groups related to phenols, amines, hydrocarbons, ketones and alkenes in both extracts. This confirms the rich chemical composition of *R. arboreum*, contributing to its potential therapeutic properties.

NMR analysis of *R. arboreum* leaves and flowers extract revealed various proton environments, indicating a complex molecular structure. The presence of deshielded protons suggests vinylic or aromatic groups, while peaks associated with protons adjacent to electronegative atoms or carbonyls indicate functional groups like ethers or alcohols. A significant presence of aliphatic protons was also noted, suggesting long-chain aliphatic compounds. The observed patterns imply the molecule contains multiple methyl and methylene groups, highlighting its potential complexity.

GC-MS analysis of the crude extract from *R. arboreum* leaves and flowers identified a total of 135 compounds in the leaves and 184 in the flowers, excluding derivatives and concentrating on specific interests. Distinct peaks for each component were detected through chromatography, with each peak corresponding to a specific compound. The identification of these compounds was accomplished by analyzing the retention time and mass spectra linked to the peaks.

PHASE II

The present study evaluated the radical scavenging activity of leaves and flowers extract of *R. arboreum* using the DPPH assay. DPPH showed that the IC_{50} values for *R. arboreum* leaves and flowers extract were 56 µg/mL and 67 µg/mL, respectively. These results indicate potent radical scavenging activity for both extracts.

The methanol extract of *R. arboreum* leaves and flowers exhibited an IC_{50} value of 60 µg/mL and 65 µg/mL in the ABTS assay. These results indicated variability in radical scavenging activity between the leaves and flowers.

In the hydrogen peroxide scavenging assay, the IC_{50} values for the methanol leaves extract were found to be 53 µg/mL, while the flowers extract showed an IC_{50} value of 57 µg/mL. These results indicated that both extracts possess significant antioxidant potential against hydrogen peroxide-induced oxidative stress.

In the lipid peroxidation inhibition (LPO) assay, the IC_{50} values for methanol extracts from the leaves and flowers of *R. arboreum* were 56.6 µg/mL and 60.4 µg/mL respectively, demonstrating significantly higher inhibition compared to ascorbic acid alone ($p < 0.01$) tested in egg yolk. The antioxidant efficacy of *R. arboreum* extracts emphasizes the potential therapeutic value in mitigating oxidative damage related to lipid peroxidation.

The Ferric Reducing Antioxidant Power (FRAP) assay for *R. arboreum* flowers and leaves extract revealed a clear trend in antioxidant activity across varying

concentrations (0 to 250 µg/mL). Both the extracts showed an increasing FRAP values with higher concentrations, indicating enhanced reducing power.

The crude extracts of *R. arboreum* flowers and leaves were dissolved in saline water to prepare a 1mg/mL stock solution. Different volumes (100, 250, 500, 1000, 1500 µL) of the extracts were added to saline solution for the brine shrimp lethality bioassay. Thirty shrimps were introduced to each concentration, with brine solution as a blank and potassium dichromate (1mg/mL) as a positive control. Shrimp mortality was monitored over 24 hours, and both the extracts showed significantly lower toxicity compared to potassium dichromate, with only 1 to 13 mortalities at the highest concentrations.

The cytotoxic activity of *R. arboreum* leaves and flowers methanol extract. showed the strong cytotoxicity, with inhibition ranging from 43.5% at 125 µg/mL to 66.2% at 1000 µg/mL for leaves, while the flowers extract exhibited inhibition between 46.7% and 59.9% at the same concentrations. The IC_{50} values were 0.541 mg/mL for flowers extract and 0.841 mg/mL for leaves extract. Flow cytometry analysis further confirmed a higher rate of apoptosis in cells treated with flowers extract when compared with leaves extract. Both extracts induced apoptosis in a concentration-dependent manner, highlighting their cytotoxic potential.

PHASE III

In this study, forty C57BL/6 mice (25–30 g) from Biogen, Bangalore, were housed under controlled conditions. The experiment, approved by IAEC (Ethical Approval No. AIW: IAEC.2023:11), involved seven groups. Group I was the control and Group II received AGS cells. Groups III to VII received AGS cells and treatments (standard drug and plant extracts) at varying doses. After 8 weeks, tumor analysis was conducted, including stomach homogenization and centrifugation. The supernatant was collected for *in vivo* studies. Initial body weights averaged 20.6±1.86 g in Group I, with changes observed across treatment groups. Stomach weight varied with treatments.

Tumor development was observed at a 200 mg/kg dose combined with AGS induction. *R. arboreum* methanol flowers and leaves extract were tested for anti-hematologic neoplasm effects. Groups II, VI, and VII showed increased RBC counts, while WBC and lymphocyte counts remained similar. Hemoglobin levels increased in Groups VI and VII. Serum markers (urea, uric acid, creatinine) indicated kidney stress.

Antioxidative effects were observed, especially at 200 mg/kg, reducing tumor incidence and increasing body weight. Lipid peroxidation was elevated in Group VI. Antioxidant enzyme activities (SOD, CAT, GPx, GSH) were lower in cancer-induced

mice but improved with *R. arboreum* leaves high dose treatment ($P < 0.001$). Myeloperoxidase (MPO) activity was higher in Group II (untreated) compared to all other treatment groups.

Histological analysis of the Group I (control) revealed a normal gastroesophageal junction with consistent non-neoplastic gastric foveolar and squamous epithelium, showing no abnormalities across the stomach layers. In contrast, Group II (untreated) exhibited lesions lined by low-grade neoplastic columnar epithelium with gastric glands containing paneth and goblet cells, while Group III (docetaxel) displayed a non-neoplastic gastric columnar lining epithelium with edematous submucosa. In Groups IV (low dose) and V (high dose) *R. arboreum* flower extracts, the microscopic analysis revealed a gastroesophageal junction with non-neoplastic gastric columnar lining epithelium. Groups VI (low dose) and VII (high dose) *R. arboreum* leaf extracts showed non-neoplastic gastric foveolar epithelium folded and lined by columnar epithelium, indicating a complete response to treatment.

The flower extract-treated groups demonstrated limited immune cell infiltration in the tumor microenvironment, with scattered lymphocytes, macrophages and neutrophils, suggesting a subdued immune response and limited immunomodulatory capacity. On the other hand, leaf extract treatment showed a complete response with well-organized foveolar and mucosal cells, indicating restoration of normal gastric epithelium and highlighting its potent therapeutic potential in reversing tumor-associated damage. Both the extracts demonstrated cytoprotective effects, such as reduced ulcer areas, decreased edema and leukocyte infiltration, particularly at a 200 mg/kg body weight dosage combined with AGS induction.

PHASE IV

Ten key chemical compounds from the leaves and flowers of *R. arboreum* were identified, including beta-sitosterol and quercetin. These compounds were mapped to their respective human gene targets, with 958 gastric cancer-related targets identified. A comparison revealed 115 unique targets, with 22 common targets selected for further study.

Enrichment analysis identified key pathways, including EGFR tyrosine kinase inhibitor resistance, and highlighted significant genes like EGFR, ESR1 and MMP9. The molecular docking study assessed the binding affinities of five compounds beta-sitosterol, quercetin, gamma-sitosterol, alpha-amyrin, and 2,3,4,6-tetramethoxystyrene against four key target proteins (PDB IDs: 1B3D, 1ERR, 6GQO, and 6S9B). Quercetin

exhibited the highest binding affinities, with energies of -8.68 kcal/mol for 1B3D, -6.407 kcal/mol for 6S9B, -6.053 kcal/mol for 6GQO and -5.374 kcal/mol for 1ERR.

The docking conformations of critical regulatory targets ESR1, KDR, MMP9, and EGFR identified through KEGG analysis were evaluated. These results suggested quercetin as a promising candidate for further investigation due to its strong interactions with above mentioned proteins. The present study conducted molecular docking of ligands and standard drug-docetaxel with gastric cancer-related proteins (MMP9, EGFR, KDR, and ESR1) to assess binding affinities, which are critical for tumor invasion and progression. The results were compared with bioactive ligands of *R. arboreum* extracts to evaluate the therapeutic potential and mechanisms of both docetaxel and the natural compounds in inhibiting gastric cancer pathways.

The molecular docking results indicated that quercetin exhibited the highest binding affinity against MMP9 (PDB ID: 1B3D) with a score of -8.68 kcal/mol, surpassing docetaxel's score of -5.318 kcal/mol. This suggested quercetin may offer better inhibition potential for MMP9. Other ligands, such as gamma-sitosterol (-4.53 kcal/mol), beta-sitosterol (-4.36 kcal/mol), and 2,3,4,6-tetramethoxystyrene (-4.23 kcal/mol), exhibited moderate affinities, while alpha-amyrin had the weakest affinity (-3.56 kcal/mol), still comparable to docetaxel.

For EGFR (PDB ID: 6S9B), quercetin again showed the strongest binding affinity at -6.40 kcal/mol, outperforming docetaxel (-3.783 kcal/mol). Other ligands, including 2,3,4,6-tetramethoxystyrene (-3.29 kcal/mol), beta-sitosterol (-2.67 kcal/mol), and gamma-sitosterol (-1.31 kcal/mol), displayed weaker interactions, with alpha-amyrin showing the lowest score (-1.16 kcal/mol). In KDR protein (PDB ID: 6GQO), quercetin maintained the score of -6.05 kcal/mol, whereas docetaxel showed binding affinities of -6.621 kcal/mol. Other ligands had lower affinities: gamma-sitosterol (-3.05 kcal/mol), beta-sitosterol (-3.04 kcal/mol) and alpha-amyrin (-3.01 kcal/mol).

Lastly, for ESR1 (PDB ID: 1ERR), quercetin showed the highest binding affinity at -5.37 kcal/mol, slightly lower than docetaxel (-5.698 kcal/mol). Other ligands, including 2,3,4,6-tetramethoxystyrene (-3.28 kcal/mol), beta-sitosterol (-3.05 kcal/mol), and alpha-amyrin (-2.62 kcal/mol), exhibited moderate binding, while gamma-sitosterol had the lowest affinity (-2.52 kcal/mol).

Molecular dynamics simulations (MDS) were performed over approximately 100 nanoseconds to assess the stability of protein-ligand complexes under varying physiological conditions. The Root Mean Square Deviation (RMSD) analysis indicated

distinct fluctuations and stabilization patterns across different protein-ligand complexes. For complex 1B3D, the first fluctuation occurred at 13.10 ns, with protein and ligand RMSD values of 2.47 Å and 1.53 Å, respectively. Complex 6S9B exhibited fluctuations starting at 4.00 ns, reaching higher RMSD values (protein: 2.77 Å, ligand: 4.14 Å) before stabilizing at 14.10 ns. The 6GQO complex showed a significant initial ligand RMSD of 13.32 Å at 4.30 ns, which was stabilized in 5.54 Å at 40.50 ns. In the case of 1ERR, fluctuations began at 3.40 ns, with subsequent stabilization and later fluctuations at 63.80 ns.

Root Mean Square Fluctuations (RMSF) analysis revealed varying degrees of flexibility in both protein chains, indicating dynamic structural changes. The ligand properties of quercetin, including radius of gyration, RMSD, intramolecular hydrogen bonds, molecular surface area, solvent accessible surface area and polar surface area, remained stable throughout the simulations, suggesting quercetin's suitability for drug discovery. Overall, the simulations confirmed quercetin's superior binding energy compared to other compounds, underscoring its potential role in modulating gastric cancer pathogenesis through key target proteins.

SUMMARY AND CONCLUSION

Rhododendron arboreum, traditionally used in medicine, has gained attention for its phytochemical properties and potential anti-cancer activity. This study conducted qualitative and quantitative analyses of leaves and flowers, revealing diverse bioactive compounds in methanol extract. Qualitative analysis confirmed alkaloids, carbohydrates, flavonoids and phytosterols in the leaves, while flowers contained amino acids, diterpenes and coumarins. Quantitative findings indicated high flavonoid concentrations 117.1 mg/g in leaves and 139.6 mg/g in flowers underscoring the plant's therapeutic potential. Antioxidant assays, including DPPH, ABTS and FRAP, demonstrated significant free radical scavenging activity, with leaf extract showing superior efficacy. The brine shrimp lethality assay revealed low toxicity for methanol extracts, while the MTT assay indicated that leaf extract exhibited the highest cytotoxicity against AGS gastric cancer cells. In a C57BL/6 mice model, treatment with *R. arboreum* extracts stabilized body weight and influenced organ weights, suggesting a protective effect against tumor burden. Hematological evaluations showed increased red blood cell counts and total hemoglobin levels in treated groups, indicating enhanced erythropoiesis.

Biochemical analyses highlighted altered liver enzyme levels, warranting further investigation. GC-MS and IMPACT database identified ten key compounds, including quercetin and beta-sitosterol, linked to 115 unique gastric cancer targets. Pathway enrichment analyses underscored the significance of genes like EGFR and MMP9 in cancer pathways. Molecular docking studies identified quercetin as the most promising compound, demonstrating high binding affinities to critical target proteins and suggesting its potential as an effective anti-cancer agent.

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